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TAXONOMY AND BIOLOGY OF PHLEBOTOMINE VECTORS OF HUMAN DISEASE

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A brief review of taxonomic criteria and new methods in phlebotomine taxonomy, including computer-assisted identification, was completed. Collaborative studies on sand fly genetics concentrated on karyotyping and isozyme analysis and were found useful in evaluating variation. Holotypes of Lutzomyia stored in France and many other specimens were examined to help complete a review of the neotropical species in this genus. Biological/disease studies were limited to: 1) dispersal studies of Lutzomyia spp. in Colombia; 2) evaluating the effects of oostatic hormone on female L. anthophora; and 3) studying the development of a new Leishmania sp. in experimentally-infected L. trapidoi.

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PROGRESS REPORT

Introduction

This report covers the period from 31 Dec. 1986 to 31 Dec. 1987. Research was focused mainly on New World sand flies with the aim of completing a handbook on the Neotropical fauna.

Objectives

1. To study the taxonomy of sand flies from the Neotropical Region, with special emphasis on the fauna of Mexico and Central America, and to provide a handbook on the entire fauna.
2. To study the taxonomy of sand flies from Africa and the Near East, providing identification keys, reference collections (based mainly on newly collected material); and to evaluate future needs for study in these areas.
3. To colonize, in the laboratory, different species of Phlebotominae from Africa, the Near East and the Neotropical Region, with special emphasis on vector species.
4. To assess the susceptibility of colonized species to aetiologic agents of disease.

Results

Taxonomic studies of Phlebotominae

1. A review of recent advances and methods in phlebotomine taxonomy was completed (Appendix I). This contribution will be published in a forthcoming book sponsored by the Canadian government and the UNDP/World Bank/WHO Special Programme from Research and Training in Tropical Diseases.

2. The PI collaborated for one month with two French scientists in compiling and writing a computer-aided identification manual to the 70+ *Lutzomyia* species of French Guiana. An associated software program was also created that will assist users in determining unknown specimens more rapidly than with traditional identification keys. The program and manual are now available (J. Lebbe et al. 1987. Computer aided identification of phlebotomine sandflies of French Guiana. Institut Pasteur de la Guyane Francaise, 165 pp).

3. Genetic studies of sand flies, in collaboration with Dr. R.D. Kreutzer, continued in 1987. Brain cell karyotypes of eight *Lutzomyia* species have been determined, six of which were studied during 1987. Variation in chromosome number was noted only in *Lutzomyia trapidoi* (2n = 6). The remaining seven species, including *L. gomeizi* and *L. longipalpis*, have 8 chromosomes (2n = 8). Structural variation of the chromosomes will be shown and discussed in a paper accepted by the Journal of Medical Entomology for publication (Kreutzer et al. 1988. Brain cell karyotypes of six New World sand flies).

Isozyme data were used to study the phylogenetic relationships of 10 Lutzomyia species in the medically important verrucarum species group. Three taxa, L. longiflocosa, L. sauroida and L. quasitownsendi, are probably conspecific based on the statistical values of genetic identity (I) and distance (D). Electrophoretic data were also useful in separating isomorphic females in this complex. Specific information will be given in a paper (Kreutzer et al. 1988. Genetic relationships among phlebotomine sand flies J. Med. Entomol.) that will be submitted for publication in 1988.

4. Holotypes of New World phlebotomines housed at the Institute Pasteur, Paris, were examined and illustrated. Several taxa are known only from single specimens -- L. elongata, L. quadrispinosa and L. sylvicola -- and, therefore, it was important to study these specimens. The first draft of a review of the Lutzomyia species in Mexico, Central and South America was completed. Some additional information will be added to this manuscript following a trip to Peru (Feb. 1988) where holotypes of several rare Peruvian phlebotomines are kept. At present, the manuscript contains more than 1000 pages, including distribution maps and figures.

5. Results of a mark/recapture study of phlebotomines in a Colombian leishmaniasis focus were published in 1987.

6. Several thousand specimens of *Lutzomyia*, *Sergentomyia*, *Phlebotomus* and *Brumptomyia* were received from colleagues working in Kenya, Guatemala, Venezuela, Colombia and Brazil. These will be incorporated into the reference collection following identification and slide mounting.

7. A laboratory colony of *Lutzomyia anthrophora* is being maintained at the University of Florida for use in studies related to age grading of male and female sand flies and to evaluate the effects of oostatic hormone, a newly discovered peptide that inhibits blood digestion in blood feeding Diptera. Preliminary studies indicate this hormone, when injected into fully fed *L. anthophora*, is effective in preventing egg development due to inhibition of blood digestion.

8. Several hundred F_1 progeny of *L. trapidoi*, collected in Colombia by the PI and colleagues, were experimentally infected with an undescribed *Leishmania* to study its extrinsic development. This parasite belongs in the *braziliensis* complex and has been isolated from sand flies and human patients in Colombia and Panama, as well as sloths in the latter country.

APPENDIX I

TAXONOMIC CRITERIA AND NEW METHODS IN PHLEBOTOMINE IDENTIFICATION

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Taxonomic research on phlebotomine sandflies requires no justification when viewed in relation to epidemiological investigations of the leishmaniases. Because of their role as vectors, these insects are better known than the nonbiting psychodids in other subfamilies. Present knowledge of the diversity of sandfly taxa is based largely on the morphology of the adults, especially after 1926 when Adler and Theodor (1) first pointed out the utility of the female spermathecae and cibaria as diagnostic characters. These and other structures have also provided a basis for grouping sandflies into supraspecific categories, the number and concepts of which vary according to author (22).

In the present paper, we are more concerned with the criteria and methods used in identifying species and subspecies of Phlebotominae. Examples are given for members of *Phlebotomus* and *Lutzomyia*, the two genera that contain proven vectors of leishmaniasis in the Old and New Worlds, respectively. More than 500 species have been described in these two categories (21, 23).

Species Definition

Following the biological species concept, a sandfly species may be defined as a "group of interbreeding natural populations that are reproductively isolated from any other such groups" (3). It is necessary to apply one or more criteria to determine the taxonomic status of individuals in these populations.

Morphology And Coloration Of Sandflies

Classical taxonomy has relied heavily on adult morphology and, to a lesser extent, external coloration to distinguish extant and extinct phlebotomines. General taxonomic reviews of *Phlebotomus* (2, 21) and *Lutzomyia* (13, 23) illustrate the importance of these critera. Nevertheless, slight or no discernible changes in the phenotype of an undetermined number of species occurred during speciation and there is a broad spectrum of structural diversity among these insects, including vectors.

At one extreme, it is likely that different species exist that are morphologically indistinguishable in both sexes. Preliminary studies of two populations of *P. ariasi* in France (34) indicate, for the first time, that such species pairs may occur in Phlebotominae. Differences in behavior, ecology, and physiology of individuals in sympatric populations provide clues suggesting that two or more distinct taxa are present.

There are reports of other sibling species or morphospecies in the subfamily that can be structurally distinguished with certainty in one sex or the other, usually the male. Examples among sympatric vectors include *Lu. wellcomei* and *Lu. complexa* in Brazil (18) and *P. martini*, *P. celiae* and *P. vansomeranae* in Kenya (21).

Attempts to differentiate morphospecies by structure or other means begin with examining known individuals of each species from individual rearings in the laboratory or by obtaining wild caught specimens in localities where no more than one morphospecies exists. Close comparisons of this material may lead to previously overlooked structural differences such as the relative lengths of the antennal ascoids, recently shown to be reliable for separating *P. papatasi* and *P. bergeroti* females (17). The use of detailed morphometric analysis (11) was first applied to sandfly taxonomy by Lane & Ready (18) who attempted to distinguish females of *Lu. wellcomei* and *Lu. complexa*. Not all females could be determined by these methods but their use should be expanded to include other species complexes.

New advances in rearing phlebotomines (15, 26) will no doubt aid these and other studies pertaining to the morphology and number of chromosomes. There is a paucity of data on cytogenetics of sandflies. Examination of the polytene chromosomes in the salivary glands of larvae (39) and of brain cells (16; R. Kreutzer, unpub. data) has shown that the chromosome number varies according to species (e.g., 2N = 6 for *P. argentipes*, *P. colabensis*, *Lu. trapidoi*; 2N = 8 for *Lu. longipalpis*, *P. papatasi*; 2N = 10 for *P. perniciosus*).

The intensity and/or distribution of external pigmentation may vary interspecifically and can be used in species diagnosis. The discovery of dark specimens of "*Lu. carrerai*" in parts of Bolivia (6), Peru (42), and Brazil (J. Arias, pers. comm.) prompted other studies that confirmed the hypothesis that the dark individuals were not conspecific with that species. Further examination showed that the dark species, *Lu. yucumensis*, could be separated by slight, but consistent, structural differences (6). Coloration alone is used to distinguish males of *Lu. shawi* from those of *Lu.*

richardwardi in Brazil (31) and provides an easy way to distinguish one-spot and two-spot forms of *Lu. longipalpis* males (38), the status of which has not been resolved.

Variation in coloration or structure between individuals of allopatric populations, such as the one- or two-spot forms of *Lu. longipalpis*, can be problematical when the differences are minor. Additional information from cross-breeding experiments (38), behavior, electrophoresis, and other studies may help shed light on the taxonomy of such forms.

Nomenclatural decisions regarding the status of these variants are somewhat subjective without additional knowledge. Some vectors such as *Lu. olmeca* in the neotropics are regarded as polytypic, with two or more named subspecies (41). In contract, *P. langeroni* and *P. orientalis* and several species in the *verrucarum* group related to *Lu. townsendi* are treated as distinct species (21, 12). The names used for these taxa, whether specific or subspecific, are not especially important from the epidemiologic standpoint as long as the distinguishing features are unambiguous and the individuals can be correctly identified.

Biochemical Methods In Taxonomy

Enzyme Electrophoresis

Buth (5) reviewed electrophoresis data in relation to biosystematics in general, but the concepts and analysis apply to phlebotomine taxonomy as well. Enzyme electrophoresis for estimating genetic variation was first applied to insects (*Drosophila*) in the late 1960's (3) and to phlebotomines about a decade later (25). Enzyme variants may be species-diagnostic and are therefore useful for detecting or distinguishing sibling species. For example, diagnostic enzyme profiles served to separate *Lu. carrerai* and *Lu.*

yucumensis -- two morphologically similar species in Bolivia (6). Ready & Silva (32), on the other hand, did not find enzyme variants (allozymes) that could distinguish *Lu. wellcomei* from *Lu. complexa* females in Brazil but only 11 enzyme systems could be successfully resolved. Infraspecific variation of some sandflies has also been investigated or revealed by electrophoretic data (4, 24, 29, 35, 36, 37). An increasing number of laboratories in endemic areas of leishmaniasis are using these methods to characterize *Leishmania* and it would take relatively little effort and expense to use the same methods for phlebotomines.

Cuticular Hydrocarbon Analysis (CHA)

Cuticular hydrocarbon analysis, introduced as a taxonomic tool for studying vectors in 1979 (7), has been used to distinguish adults of sibling phlebotomines in Brazil (33) and two populations of *P. ariasi* in France (34). In the latter study, it was shown that the CH profiles of *P. ariasi* larvae differed from those of conspecific adults. Sandflies collected in the field can be stored dry at ambient temperatures until processed (30). Moreover, the technique, unlike that of enzyme electrophoresis, does not destroy the insects so that voucher specimens can be saved for additional taxonomic studies.

DNA Probes

New technology in developing specific DNA probes for distinguishing organisms, including insects (8, 9, 14), has not yet been applied to phlebotomines. A probe for identifying females or males of the *Anopheles gambiae* complex is used routinely in one laboratory (9). DNA, extracted from whole or parts of mosquitoes, provides sufficient material for assays.

Preliminary data suggest that the probe is equally effective for immature stages as well (9).

Computer Assisted Taxonomy

Analyzing data from taxonomic studies is efficiently accomplished with the aid of computer-based technology. It is possible to accurately measure the size and shape of insect structures with computers and electronic measuring equipment (11). There are many statistical programs available for microcomputers that are now available in most parts of the world. Furthermore, computer-aided identification programs are available (e.g., 10, 19, 27, 28, 40), one of which allows the user to identify sandflies of French Guiana (20). Such a program, when broadened geographically, would be an excellent method for training nonspecialists in sandfly identification.

Concluding Remarks

The methods and criteria used in sandfly systematics continue to change as new information becomes available. The search for new taxonomic characters and new ways of analyzing known characters that distinguish taxa and show relationships among them, is an important continuing objective in biosystematics. Computer-aided taxonomy will no doubt play a more important role in the future but classical methods will not become obsolete.

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